ISSN No:-2456-2165

GC-MS Analysis of *Nigella Sativa* Seed Extract and Its Ameliorative Effects on Transgenic *Drosophila* Model of Parkinson Disease

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Abstract:- Bioactive compounds in plants, referred to as secondary metabolites, can impact the physiological and cellular activities of living organisms. A systematic screening of plant species to discover new bioactive compounds is essential for any bioprospecting study. Parkinson's disease is the most common neurodegenerative disease characterized by the degeneration of dopaminergic neurons in substantia nigra par compacta and alpha synuclein aggregation within the brain. *Drosophila* has been intensely used as a genetic model system, for its ease in laboratory maintenance and genetic manipulation. Due to the advantages of *Drosophila* model system, it is used to asses lifespan expansion, behaviour studies and neuroprotective activities of plant extracts to counteract PD symptoms. The present study intended to identify the bioactive constituents present within the ethanolic extract of *Nigella sativa* and evaluate its neuroprotective effects on *Drosophila* expressing normal human alpha synuclein (h-αS) in the dopaminergic neurons. As the age progresses, these flies exhibit short lifespan and locomotor dysfunction. Results of this study exhibit the dose-dependent delay in PD flies and an increase in their lifespan. Gas chromatography- Mass spectrometry (GC-MS) analysis *Nigella sativa* seed extract confirmed the presence of 51biologically active phytocompounds in the above extract. The present study demonstrates the potency of *Nigella sativa* seed extract in delaying the PD symptoms in PD model flies. Further studies are required to isolate the bioactive compounds and review the precise mechanism of action in various diseases.

I. INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disease identified by the degeneration of dopaminergic neurons in the substantia par compacta (SNpc) region and characterized by the presence of Lewy bodies and Lewy neurites [1, 2]. PD is the second largest neurodegenerative disease which affects ~1% of the population at the age of 65 [3]. Cardinal signs of PD include resting tremors, rigidity, postural instability and secondary manifestations such as dementia, soft speech and difficulty in swallowing due to uncoordinated mouth and throat movement. Adverse environmental and genetic factors are known to exacerbate and advance the development of PD [4, 5]. Although the precise mechanism of neurodegeneration in SNpc is elusive, the cascade of events such as oxidative stress, dysfunction of and ubiquitin-proteasome system, dopamine auto-oxidation, α-synuclein aggregation, glial cell activation, alterations in calcium signaling and excess free iron have also been associated with PD. Further, several molecular mechanisms are known to trigger dopamine neuron death. However, these molecular mechanisms triggering the dopamine neuron death have remained poorly understood. Studies have shown dopamine supplements to enhance the recovery by reversing the symptoms and restoring the levels of dopamine. However, long-term dopamine usage leads to the physiological adaptations rendering its usage nonfunctional/ inutile and is regarded to have side effects [6, 7].

Drosophila has emerged as an excellent model for understanding the mechanism involved in neurodegenerative disorders. Genomic studies have shown about 75% of genes in *Drosophila* is orthologs for human disease. *Drosophila* has a relatively simple central nervous system and lacks an endogenous α-synuclein gene or ortholog. However, Feany and Bender have shown that both wild type and mutated (A30P or A53T) flies shows symptoms similar to those found in human PD patients. The yeast-based UAS (upstream activating sequence) - GAL4 system is an effective bipartite system that enables gene expression activation in *Drosophila* [8]. Furthermore, *Drosophila* is extensively employed for the assessment of standard neurodegenerative disease, treatment and for screening therapeutic agents to study their effects on biological, biochemical, and genetic processes [9, 2].

For thousands of years, plants have been used as a major source of medicinal treatments. Plant derived products play a very vital role in the primary health care of approximately 80% of the world population and it is important to conduct studies to confirm the ethnomedicinal use of such herbal plants. Furthermore, isolation and identification of compounds will likely add value to the drug. The use of traditional medicine is encouraged by the World Health Organization (WHO), provided they are safe and have fewer side effects than synthetic medicines [10]. The bioactive compounds derived from plants, referred to as phytochemical compounds are produced as secondary metabolites and are regarded to elicit pharmacological or effects in living organisms [11].

Nigella sativa L commonly known as Black cumin seeds is an indigenous herb belonging to the family Ranunculaceae, cultivated in India, the Middle East, Northern Africa, Mediterranean countries, Eastern Asia and Eastern Europe. For centuries these seeds have been used in traditional medicine for treating numerous disorders [12]. *N.sativa* seeds are extensively studied due to their various pharmacological and biological uses. Traditionally these seeds have found to be effective for treating various ailments. Since it is regarded to have properties such as stomachic, immunomodulatory and digestive properties, they also possess anti-cancer, anti-inflammatory, anti-microbial, anti-tumor and antioxidant activities [13].The seeds of *N.sativa* constitutes carbohydrate 35%, proteins 22.7% and plant oil and fat 35-38% and also contain fiber, minerals like Fe, Na, Cu, Zn, P, Ca and vitamins like vitamin C, thiamine, niacin, pyridoxine and folic acid [14, 15]. Recent studies have shown *N*.sativa to have various pharmacologically active compounds like thymoquinone which attains up to 27.8% of the volatile oil (w/w), Carvacrol 5.8-11.6% (w/w), p-cymene 15.5-31.7% (w/w), alpha-pinene 9.3%, 4-terpineol 2-6.6%, and longifolene 1-8% (w/w), t-anethole benzene 0.25-2.3% (w/w). Other functional components include thymohydroquinone (THQ), dihydrothymoquinone (DHTQ), α-thujene, thymol, t-anethole, β-pinene, α-pinene, and γ-terpinene [16, 17]. In this present study, the transgenic flies expressing the human alpha synuclein under the UAS-GAL4 system was used to study the neuroprotective effect of *Nigella sativa* extract [18].

II. MATERIAL AND METHODS.

2.1. Collection of plant material.

Seeds of *N. sativa* were procured from local market in Bangalore, Karnataka, India, washed thoroughly and dried at ambient temperature.

2.2. Preparation of Extract.

Dried seeds of *N.sativa* were grounded to a fine powder with the aid of an electric grinder. Approximately 25g of this powdered seeds were macerated using 100 ml ethanol as a solvent for 1×24 h. The extract was then filtered, and evaporated using rotavapour. The concentrated extract thus obtained was preserved at 4°C for later use [19].

2.3. Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

Analysis of ethanolic extract of *N.sativa* was performed using GC/MS –QP2 Perkin Elmer, attached with a capillary column Restek5, RTx^{R-} (30m ×0.25mm). The extract (1mg/mL) was reconstituted in methanol; 1µL of this extract was injected with a split ratio of 20:1, as carrier gas (Helium gas (99.9%) was used at a flow rate of 1mL/min. The injector was operated at 250°C and the oven temperature was initially set to 60°C for 5 min, then the temperature was gradually increased to 280°C for 10 min. Identification of compounds was based on spectral configurations obtained with the available mass spectral database (NIST and WILLEY library) attached to the GC-MS [20].

2.4. *Drosophila* strains.

Two strains of transgenic flies of genotypes w1118; P {DdcGal4.L}4.3D were obtained from National Center for Biological Sciences (NCBS), Bangalore [(Bloom No. 7010) and w⁄; P{UAS-Hsap/SNCA.A30P}40.1 (Bloom No. 8147)].

2.5. *Drosophila* culture and Crosses.

To obtain PD model flies, males from UAS-A30P were crossed with the virgin female carrying the driver elavGal4. The F1 progeny were collected and transferred into fresh culture media and maintained under a 12:12 light-dark cycle at 25˚C and 70% humidity incubator. The PD flies were supplemented with different concentration ethanolic extract of N.*sativa* seed viz, 0.01%, 0.05%, 0.1%, and 0.5% (w/v). For the study, only male flies were chosen, and UAS-A30P was used as control [4].

2.6. *Drosophila* Lifespan Determination.

Life span of the flies were assessed using the protocol described by (Kim *et al.*, 2011), newly eclosed flies were transferred in to culture vials (10 flies/vial) containing different concentrations of *N.sativa* mixed within the diet. PD and control flies were cultured separately with and without extract. Care was taken to change the culture media on every 3rd day and number of dead flies was noted at regular interval of 3 days until the last fly was dead. 20 flies from each group in 5 replicates were used to assess the lifespan [21].

2.7. *Drosophila* Climbing Assay.

Negative geotaxis or climbing assay was used to assess locomotory functions. 10 flies were placed in an empty plastic vial (10.5 cm 2.5 cm), and a horizontal line was marked 8cm above the bottom of the vial. After 10 min, the flies were softly tapped down to the bottom of the vial and were allowed to climb up. The total numbers of flies climbed to the 8cm mark within 10s were recorded. Experiment was repeated for five consecutive times, and data recorded was expressed as mean of five trails. All behavioural studies were performed at 25±1˚C under standard lighting conditions [22].

Statistical analysis

Statistical analysis was carried out using Graph Pad Prism 6 Software. The mean survival analysis was done by Kaplan- Meier analysis, Student “t”- test was applied to calculate the significance between the groups.

III. RESULTS

Gas chromatography-Mass spectrometry analysis of an ethanolic extract of N.*sativa* seeds showed the presence of several peaks. Chromatogram peaks were interpreted using the data base of the spectrum of known components from GC-MS library. GC-MS profiling of the extract showed the presence of 13 major compounds. The identified compounds with their Peak area (%), retention time, molecular formula and molecular weight are enlisted in Table 1, The GC-MS chromatogram showing the peak areas for various compounds are represented in Fig 1. The results of the

ISSN No:-2456-2165

climbing ability of the control group remained unchanged until 21 days. Whereas, the response of PD group was found to be significantly lowered than the control group from 12th day onwards (Fig 1. A). Based on the findings, a standard period of 21 days was chosen for subsequent treatment with different concentration of *N.sativa* extract. Climbing ability after 21 days of treatment of extract on PD group treated with concentration 0.1% and 0.5% showed significant delay in the loss of climbing ability, 0.01% and 0.05% did not show any significant loss in the climbing ability (Fig 1. B). The supplementation of 0.01%, 0.05%, 0.1% and 0.5% of *N.sativa* to PD flies showed a dose dependent significant increase in the life span of PD flies compared to that of unexposed flies (Fig 2). 0.1% and 0.5% showed significant different compared to that of diseased group.

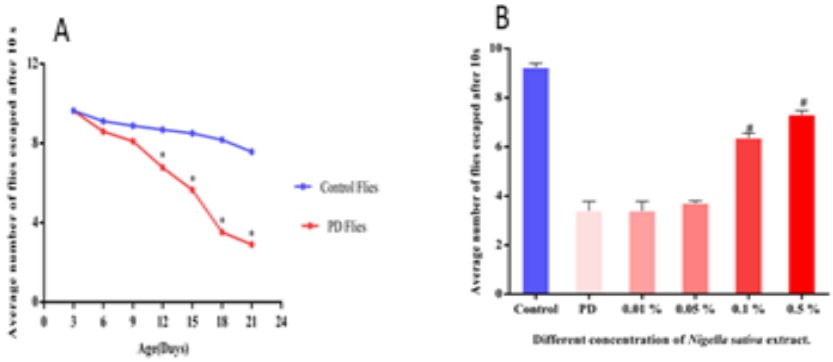

Figure 1: A. Represents the Climbing ability in Control and Parkinson's disease flies over a period of 21 days.

The values are the mean of 5 assays (* represents significance difference ($p<0.05$) compared to control flies. B. Effect of N.*sativa* on the climbing ability, the flies were subjected to climbing assay after 21 days of N.*sativa* supplementation. The values are mean of 5 assays (*significant with respect to control flies, #significant with respect to PD flies $p<0.05$).

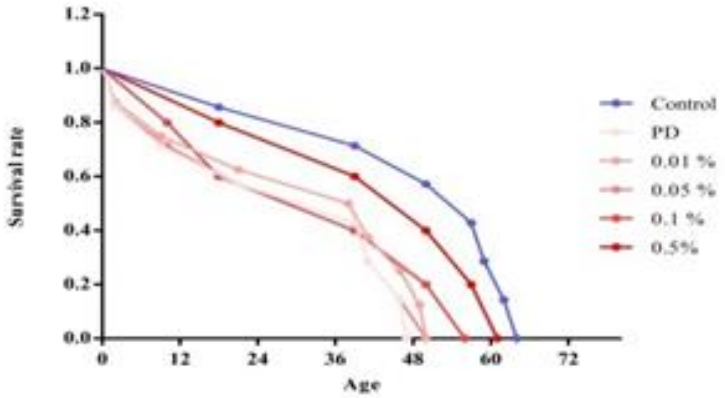

Figure 2: Effect of N.*sativa* on survival rate measured in transgenic PD flies. The values are the mean of 5 assays.

IV. DISCUSSION

Oxidative stress arising from ROS is known to cause neuronal disruption, consequently resulting in the development of neurodegenerative disorders [23]. The ability to accumulate alpha-synuclein from the wild type may contribute to fibrils formation, an insoluble protein polymer. This fibrillar alpha-synuclein along with neurofilaments and other cytoskeleton proteins are the building blocks of Lewy bodies [24]. Aggregation of these LBs leads to the accumulation of α-syn in SNpc region owing to the over production of ROS. ROS thus produce leads to the degeneration of dopaminergic neurotransmitters subsequently resulting in impairments of motor and cognitive functions. In the present study, flies expressed human α-syn under the UAS-GAL4 system mimicking neuronal degradation, memory and behavioral impairment even dopaminergic neuron death leading to the formation of Lewy bodies in a way similar to humans In substantia nigra par compacta region (17,25).

Drosophila is highly susceptible to genetic and ecological conditions. Negative geotaxis or climbing assay is a fast and easy method of determining behavioral defects and are regarded as quantitative indicator of motor abilities arising due to genetic or environmental manipulation [26]. The findings of proteomics studies have shown that more than 70-71% of the human disease-related loci have a direct ortholog in *Drosophila*. Hence, the European Centre for the Validation of Alternative Medicine (EVCAM) advocates *Drosophila* as an alternative model for experimental research and a helpful tool for pre-clinical pharmacological trials [27]. Due to the potential health benefits of plants, studies are recently being focused on biochemical screening, identification of phyto-compounds for their pharmacological uses and ameliorative role towards the neurogenerative

ISSN No:-2456-2165

diseases [28]. GC-MS analysis of *N.sativa* ethanolic extract confirmed the presence of 51 compounds (Table no 1). Several studies have confirmed the antioxidative effects of thymol and carvacrol. Furthermore, studies advocate these to have pharmacological properties such as antioxidative, anticancer, anti-inflammatory, antibacterial, antifungal, antiprotozoal, anticarcinogenic, antidiabetic, cardioprotective, and neuroprotective effects [29-31]. Longifolene is recognized to have numerous biological functions, including antifungal, antibacterial, and antioxidant potentials [31]. Likewise, α –pinene is regarded to have properties such as insecticidal, anti-carcinogenesis, anti-oxidant, and anti-inflammatory activities. Moreover, recent studies have demonstrated the anti-inflammatory activity of α-pinene through down-regulation of MAPKs (ERK and JNK) phosphorylation and NF-B signaling pathway, suggesting that α -pinene is an effective natural anti-inflammatory agent [32].

Dietary antioxidant sourced from plant extracts are considered to dramatically modulate pathways of neurodegeneration and are reported to enhance cognitive ability, mood conditions and life span in *D.melanogaster* [33-35]. Studies on fly models using dietary supplements of phytoextract have shown to ameliorate complications arising from oxidative stress and delay the loss of climbing activity (36, 37). Studies on rodent model have reported *N.sativa* oil and thymoquinone to inhibit neuronal degeneration in the hippocampal region after prolonged toluene exposure, suggesting there as anti-parkinson's property, supported by antioxidant properties [38, 39]. Thymoquinone, an important compound in *N.sativa*, is proven to have preventive effects against MPP+ and rotenone on dopamine producing cells [40]. Findings of this study have shown the ameliorative efficacy of *N.sativa* extract on Parkinson's disease and proved to improve life span, delay the loss in the climbing ability 0.1% and 0.5% showed significant improvement in locomotory behaviour, and also extend the life span when compared to diseases flies.

In conclusion, contributing to their accessibility to systematic genetic study, *Drosophila* genetic models of PD have an excellent potential for the detection of disease mechanisms. The findings of the present study suggest that PD associated motor impairments are imitated the current transgenic *Drosophila* model. It is proposed from current research that N.*sativa* extract greatly slows the deterioration of climbing ability and improves the life span. The outcome of the present study supports the fact that PD genetic model of *Drosophila* are excellent tools for evaluating potential therapeutic drugs. Further testing of N.*sativa* is required to assess the complexity involved in oxidative stress and neuro-inflammation in PD *Drosophila* models, since this analysis being the first report on the ameliorative efficacy of N.*sativa* in the PD *Drosophila* model.

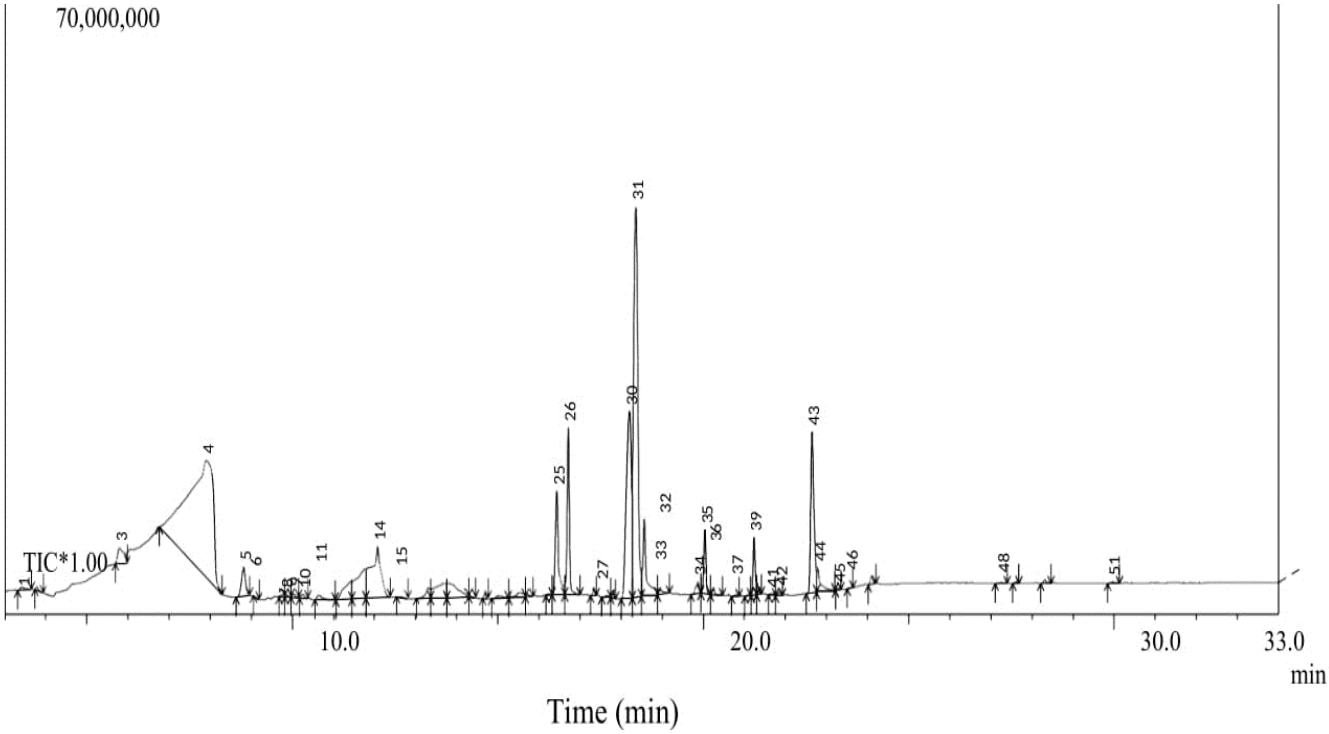

Fig 3: Chromatogram of the ethanolic extract of *N.sativa.*

ISSN No:-2456-2165

Table 1: Determination of biologically active compounds present in the ethanolic extract of *N.sativa*.

Sl. No	Compound Name	RT	Mol. Formula	Mol wt. g/mol	Peak Area %
1	2,3-Butanediol,[R-(R*,R*)]-	3.408	$C_4H_{10}O_2$	90.12	0.17
2	1,2-Cyclopentanedione	3.778	$C_5H_6O_2$	98.1	0.1
3	α-pinene	5.796	$C_{10}H_{16}$	136.23	1.11
4	Glycerene	7.902	$C_3H_8O_3$	92.09	31.56
5	o-Cymene	8.814	$C_{10}H_{14}$	134.22	1.29
6	N,N-Dimethyl-O-(1-methyl-butyl)-hydroxylamine	9.073	$C_7H_{17}N_O$	131.22	0.03
7	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	9.745	$C_6H_8O_4$	144.12	0.09
8	Phloroglucitol	9.864	$C_6H_{12}O_3$	132.16	0.08
9	3-Carene	10.03	$C_{10}H_{16}$	136.23	0.12
10	methylpropyl ester Butanoic acid, 3-oxo-, 2-	10.257	$C_8H_{14}O_3$	158.19	0.14
11	1,2,3-Benzenetriol	10.644	$C_6H_6O_3$	126.11	0.31
12	o-Isopropenyltoluene	11.428	$C_{10}H_{12}$	132.2	1.53
13	trans-4-methoxy thujane	11.767	$C_{11}H_{20}O$	168	2.93
14	1,4-Benzenediol, 2-(1,1-dimethylethyl)-	12.079	$C_{14}H_{22}O_2$	222.3	5.5
15	Heptanoic acid, propyl ester	12.602	$C_{10}H_{20}O_2$	172.2	0.05
16	2(3H)-Furanone, 5-heptyldihydro-	13.304	$C_{11}H_{20}O_2$	184.2	0.56
17	3-Deoxy-d-mannoic lactone	13.696	$C_6H_{10}O_5$	162.14	1.79
18	3 p-Dioxane-2,3-diol	13.789	$C_4H_8O_4$	120.1	1.7
19	Tetradecanoic acid	14.323	$C_{14}H_{28}O_2$	228.37	0.04
20	Tetradecanoic acid, ethyl ester	14.679	$C_{16}H_{32}O_2$	256.42	0.13
21	Methyl-diisopropylacetamide	15.005	$C_8H_{17}N_O$	143.2	0.17
22	1,5-Anhydro-d-mannitol	15.542	$C_6H_{12}O_5$	164.16	0.33
23	Pentadecanoic acid, ethyl ester	15.704	$C_{17}H_{34}O_2$	270.5	0.09
24	Thymol	16.233	$C_{10}H_{14}O$	150.22	0.04
25	l-(+)-Ascorbic acid 2,6-dihexadecanoate	16.434	$C_{38}H_{68}O_8$	652.9	3.66
26	Hexadecanoic acid, ethyl ester	16.713	$C_{18}H_{36}O_2$	284.5	4.08
27	5-Sec-butylpyrogallol	17.333	$C_{10}H_{14}O_3$	182.22	0.17
28	Heptadecanoic acid, ethyl ester	17.647	$C_{19}H_{38}O_2$	298.5	0.05
29	9 Benzofuran, 2,3-dihydro	17.804	C_8H_8O	120.15	0.14
30	9,12-Octadecadienoic acid (Z,Z)-	18.2	$C_{18}H_{32}O_2$	280.4	10.14
31	9,12-Octadecadienoic acid, ethyl ester	18.361	$C_{20}H_{36}O_2$	308.5	17.75
32	tert-Butyl-p-benzoquinone	18.563	$C_{10}H_{12}O_2$	164.2	2.88
33	2H-1-Benzopyran-5-carboxaldehyde, 3,4-dihydro-6-hydroxy-2,2,7,8-tetramethyl-	18.927	$C_9H_8O_3$	164.16	0.25
34	Indole	19.866	C_8H_7N	117.15	0.38
35	Carvacrol	20.035	$C_{10}H_{14}O$	150.22	1.58
36	Heptadecanoic acid, ethyl ester	20.251	$C_{19}H_{38}O_2$	298.5	0.17
37	10-Undecynoic acid, methyl ester	20.795	$C_{12}H_{22}O_2$	198.3	0.04
38	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	21.092	$C_{57}H_{104}O_6$	885.4	0.03
39	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	21.234	$C_{19}H_{38}O_4$	330.5	1.33
40	(+-)-3-Methyl-1-penten-3-ol	21.306	$C_6H_{12}O$	100.16	0.21
41	Linoleic acid ethyl ester	21.642	$C_{20}H_{34}O_2$	306.5	0.05
42	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.beta.,4.alpha.,5.alpha.)-	21.858	$C_{32}H_{52}O_2$	468.7	0.09
43	2-Pentylcyclopentanone	22.647	$C_{10}H_{18}O$	154.25	5.69
44	Octadecanoic acid, 2,3-dihydroxypropyl ester	22.789	$C_{21}H_{42}O_4$	358.5	0.98
45	Longifolene-(V4)	23.274	$C_{15}H_{24}$	204.3	0.03
46	Squalene	23.574	$C_{30}H_{50}$	410.7	0.02
47	10,13-Eicosadienoic acid, methyl ester	24.093	$C_{21}H_{38}O_2$	322.5	0.24
48	Campesterol	27.255	$C_{28}H_{48}O$	400.7	0.03
49	Stigmasterol	27.601	$C_{29}H_{48}O$	412.7	0.03
50	.gamma.-Sitosterol	28.313	$C_{29}H_{50}O$	414.7	0.1
51	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	29.963	$C_{30}H_{52}O_2$	444.7	0.05

ACKNOWLEDGEMENT

The author is thankful to Directorate of Minorities, Government of Karnataka, for providing partial financial support and Department of Zoology, Bangalore University, Bangalore.

Authorship Statement The experimental design of the present study and guidance was given by **Dr. Usha Anandhi. D** practical aspects, data analysis and writing were carried out by **Saliya S Bawani.**

REFERENCES

[1]. Uhl, G. R. (1990). Parkinson's disease: neurotransmitter and neurotoxin receptors and their genes. *European neurology*, *30*(Suppl. 1), 21-30.

[2]. Khotimah, H., Ali, M., Sumitro, S. B., & Widodo, M. A. (2015). Decreasing α-synuclein aggregation by methanolic extract of Centella asiatica in zebrafish Parkinson's model. *Asian Pacific Journal of Tropical Biomedicine*, *5*(11), 948-954.

[3]. Mohite, G. M., Dwivedi, S., Das, S., Kumar, R., Paluri, S., Mehra, S., ... &Maji, S. K. (2018). Parkinson's disease associated α-synuclein familial mutants promote dopaminergic neuronal death in Drosophila melanogaster. *ACS chemical neuroscience*, *9*(11), 2628-2638.

[4]. Kim, S. I., Jung, J. W., Ahn, Y. J., Restifo, L. L., & Kwon, H. W. (2011). Drosophila as a model system for studying lifespan and neuroprotective activities of plant-derived compounds. *Journal of Asia-Pacific Entomology*, *14*(4), 509-517.

[5]. Werner, F. M., & Covenas, R. (2010). Classical neurotransmitters and neuropeptides involved in major depression: a review. *International Journal of Neuroscience*, *120*(7), 455-470.

[6]. Kumar, H., Lim, H. W., More, S. V., Kim, B. W., Koppula, S., Kim, I. S., & Choi, D. K. (2012). The role of free radicals in the aging brain and Parkinson's disease: convergence and parallelism. *International Journal of Molecular Sciences*, *13*(8), 10478-10504.

[7]. Fujita, K. A., Ostaszewski, M., Matsuoka, Y., Ghosh, S., Glaab, E., Trefois, C., & Diederich, N. (2014). Integrating pathways of Parkinson's disease in a molecular interaction map. *Molecular neurobiology*, *49*(1), 88-102.

[8]. McGurk, L., Berson, A., & Bonini, N. M. (2015). Drosophila as an in vivo model for human neurodegenerative disease. *Genetics*, *201*(2), 377-402.

[9]. Caygill, E. E., & Brand, A. H. (2016). The GAL4 system: a versatile system for the manipulation and analysis of gene expression. In *Drosophila* (pp. 33-52). Humana Press, New York, NY.

[10]. Stalin, N. (2018). Screening of phytochemical and pharmacological activities of Syzygium caryophyllatum (L.) Alston. *Clinical Phytoscience*, *4*(1), 3.

[11]. Friedman, M. (2014). Chemistry and multibeneficial bioactivities of carvacrol (4-isopropyl-2-methylphenol), a component of essential oils produced by aromatic plants and spices. *Journal of agricultural and food chemistry*, *62*(31), 7652-7670.

[12]. Venkatachallam, S. K. T., Pattekhan, H., Divakar, S., & Kadimi, U. S. (2010). Chemical composition of Nigella sativa L. seed extracts obtained by supercritical carbon di oxide. *Journal of food science and technology*, *47*(6), 598-605.

[13]. Ishtiaq, S., Ashraf, M., Hayat, M. Q., & Asrar, M. (2013). Phytochemical analysis of Nigella sativa and its antibacterial activity against clinical isolates identified by ribotyping. *International Journal of Agriculture and Biology*, *15*(6).

[14]. Guler, T., & Ertas, O. N. (2006). The effect of dietary black cumin seeds (Nigella sativa L.) on the performance of broilers. *Asian-australasian journal of animal sciences*, *19*(3), 425-430.

[15]. Takruri, H. R., & Dameh, M. A. (1998). Study of the nutritional value of black cumin seeds (*Nigella sativa* L). *Journal of the Science of Food and Agriculture*, *76*(3), 404-410.

[16]. Sahak, M. K. A., Kabir, N., Abbas, G., Draman, S., Hashim, N. H., & Hasan Adli, D. S. (2016). The role of Nigella sativa and its active constituents in learning and memory. *Evidence-Based Complementary and Alternative Medicine*, *2016*.

[17]. Ahmad, A., Husain, A., Mujeeb, M., Khan, S. A., Najmi, A. K., Siddique, N. A., & Anwar, F. (2013). A review on therapeutic potential of Nigella sativa: A miracle herb. *Asian Pacific journal of tropical biomedicine*, *3*(5), 337-352.

[18]. Rizzi, G., & Tan, K. R. (2017). Dopamine and acetylcholine, a circuit point of view in Parkinson's disease. *Frontiers in neural circuits*, *11*, 110.

[19]. Gomathi, D., Kalaiselvi, M., Ravikumar, G., Devaki, K., & Uma, C. (2015). GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of Evolvulus alsinoides (L.) L. *Journal of food science and technology*, *52*(2), 1212-1217

[20]. Hadi, M. Y., Mohammed, G. J., & Hameed, I. H. (2016). Analysis of bioactive chemical compounds of Nigella sativa using gas chromatography-mass spectrometry. *Journal of Pharmacognosy and Phytotherapy*, *8*(2), 8-24.

[21]. Long, J., Gao, H., Sun, L., Liu, J., & Zhao-Wilson, X. (2009). Grape extract protects mitochondria from oxidative damage and improves locomotor dysfunction and extends lifespan in a Drosophila Parkinson's disease model. *Rejuvenation research*, *12*(5), 321-331.

[22]. Pendleton, R. G., Parvez, F., Sayed, M., & Hillman, R. (2002). Effects of pharmacological agents upon a transgenic model of Parkinson's disease in Drosophila melanogaster. *Journal of Pharmacology and Experimental Therapeutics*, *300*(1), 91-96.

[23]. Xun, Z., Sowell, R. A., Kaufman, T. C., & Clemmer, D. E. (2007). Protein expression in a Drosophila model of Parkinson's disease. *Journal of proteome research*, *6*(1), 348-357.

[24]. Hindle, J. V. (2010). Ageing, neurodegeneration and Parkinson's disease. *Age and ageing*, *39*(2), 156-161.)

[25]. Feany, M. B., & Bender, W. W. (2000). A Drosophila model of Parkinson's disease. *Nature*, *404*(6776), 394-398.
[26]. Mizuno, H., Fujikake, N., Wada, K., & Nagai, Y. (2011). α-Synuclein transgenic Drosophila as a model of Parkinson's disease and related synucleinopathies. *Parkinson's disease*, *2011*.)
[27]. Benford, D. J., Hanley, A. B., Bottrill, K., Oehlschlager, S., Balls, M., Branca, F., & Schilter, B. (2000). Biomarkers as predictive tools in toxicity testing: the report and recommendations of ECVAM workshop 40. *Alternatives to laboratory animals*, *28*(1), 119-131
[28]. Vaiserman, A. M., Lushchak, V., & Koliada, A. K. (2016). Anti-aging pharmacology: promises and pitfalls. *Ageing research reviews*, *31*, 9-35.
[29]. Zeb, A., Ullah, F., Ayaz, M., Ahmad, S., & Sadiq, A. (2017). Demonstration of biological activities of extracts from Isodon rugosus Wall. Ex Benth: Separation and identification of bioactive phytoconstituents by GC-MS analysis in the ethyl acetate extract. *BMC complementary and alternative medicine*, *17*(1), 284.
[30]. Friedman, M. (2014). Chemistry and multibeneficial bioactivities of carvacrol (4-isopropyl-2-methylphenol), a component of essential oils produced by aromatic plants and spices. *Journal of agricultural and food chemistry*, *62*(31), 7652-7670.
[31]. Memar, M. Y., Raei, P., Alizadeh, N., Aghdam, M. A., & Kafil, H. S. (2017). Carvacrol and thymol: strong antimicrobial agents against resistant isolates. *Reviews in Medical Microbiology*, *28*(2), 63-68.
[32]. Kim, D. S., Lee, H. J., Jeon, Y. D., Han, Y. H., Kee, J. Y., Kim, H. J., & Kim, S. J. (2015). Alpha-pinene exhibits anti-inflammatory activity through the suppression of MAPKs and the NF-κB pathway in mouse peritoneal macrophages. *The American journal of Chinese medicine*, *43*(04), 731-742.15
[33]. Siddique, Y. H., Mujtaba, S. F., Jyoti, S., & Naz, F. (2013). GC–MS analysis of Eucalyptus citriodora leaf extract and its role on the dietary supplementation in transgenic Drosophila model of Parkinson's disease. *Food and Chemical Toxicology*, *55*, 29-35.
[34]. Chandrashekara, K. T. & Shakarad, M. N. Aloe vera or Resveratrol Supplementation in Larval Diet Delays Adult Aging in the Fruit Fly ,. 965–971 (2011). doi:10.1093/gerona/glr103
[35]. Panchal, K., & Tiwari, A. K. (2017). Drosophila melanogaster "a potential model organism" for identification of pharmacological properties of plants/plant-derived components. *Biomedicine & Pharmacotherapy*, *89*, 1331-1345.
[36]. Jahromi, S. R., Haddadi, M., Shivanandappa, T., & Ramesh, S. R. (2015). Modulatory effect of Decalepis hamiltonii on ethanol-induced toxicity in transgenic Drosophila model of Parkinson's disease. *Neurochemistry international*, *80*, 1-6.
[37]. Siddique, Y. H., Naz, F., & Jyoti, S. (2014). Effect of curcumin on lifespan, activity pattern, oxidative stress, and apoptosis in the brains of transgenic Drosophila model of Parkinson's disease. *Biomed research international*, *2014*.
[38]. Sandhu, K. S., & Rana, A. C. (2013). Evaluation of anti-parkinson's activity of Nigella sativa (kalonji) seeds in chlorpromazine induced experimental animal model. *mortality*, *22*, 23.
[39]. Kanter, M. (2008). Nigella sativa and derived thymoquinone prevents hippocampal neurodegeneration after chronic toluene exposure in rats. *Neurochemical research*, *33*(3), 579.
[40]. Radad, K., Moldzio, R., Taha, M., & Rausch, W. D. (2009). Thymoquinone protects dopaminergic neurons against MPP+ and rotenone. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *23*(5), 696-700